



CLEAN VERSION OF THE PENDING CLAIMS

SEQUENCE AND METHOD FOR INCREASING PROTEIN EXPRESSION IN CELLULAR EXPRESSION SYSTEMS

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- Sub E1
3. (Thrice amended) A polynucleotide comprising a sequence encoding a vesicular fusion factor 2 protein (Vff2p) comprising SEQ ID NO:2 or conservative variations thereof.
4. (Thrice Amended) A polynucleotide comprising SEQ ID NO:1 or a sequence encoding SEQ ID NO:2.
5. (Amended) The polynucleotide of claim 3, wherein the protein is about 32 kD.
6. (Twice Amended) The polynucleotide of claim 3, further comprising a promoter operatively linked to the sequence encoding the Vff2p.
7. (Amended) The polynucleotide of claim 6, wherein the promoter is a promoter that functions in a host cell to direct transcription of the sequence encoding the Vff2p.
8. (Twice Amended) The polynucleotide of claim 3, further comprising a sequence encoding a heterologous target protein.
- Sub E2
9. (Amended) The polynucleotide of claim 8, further comprising a second promoter operably linked to the sequence encoding the target protein.
10. The polynucleotide of claim 9, wherein the second promoter is a promoter that functions in the host cell to direct transcription of the target protein.
11. The polynucleotide of claim 7, wherein the host cell is a yeast cell.

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12. (Four Times Amended) The polynucleotide of claim 11, wherein the yeast cell is a *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Pichia pastoris*, *Hansenula polymorpha*, or *Kluyveromyces lactis*.
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- D2
14. (Thrice amended) A polynucleotide expression vector comprising a polynucleotide encoding a Vff2p comprising SEQ ID NO:2 or conservative variations thereof.

15. (Thrice Amended) An expression vector [of claim 14,] comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.

16. (Amended) The expression vector of claim 14, wherein the protein is about 32 kD.

17. (Twice Amended) The expression vector of claim 14, further comprising a promoter sequence operatively linked to the sequence encoding the Vff2p.

18. (Amended) The expression vector of claim 17 wherein the promoter is a promoter that functions in a host cell to direct transcription of the sequence encoding the Vff2p.

19. (Twice Amended) The expression vector of claim 14, further comprising a sequence encoding a heterologous target protein.

20. The expression vector of claim 19, wherein transcription of the target protein is directed by a second promoter.

21. The expression vector of claim 20, wherein the second promoter is a promoter that functions in the host cell to direct transcription of the target protein.

22. The expression vector of claim 18, wherein the host cell is a yeast cell.

23. (Amended) The expression vector of claim 22, wherein the yeast is *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Pichia pastoris*, *Hansenula polymorpha*, or *Kluyveromyces lactis*.

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25. (Twice Amended) A recombinant host cell comprising a yeast cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a functional Vff2p, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof.

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26. (Thrice Amended) A host cell comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.

27. (Amended) The host cell of claim 25, further comprising a sequence encoding a heterologous target protein.

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29. (Amended) The host cell of claim 25, wherein the yeast cell is a *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Pichia pastoris*, *Hansenula polymorpha*, or *Kluyveromyces lactis* cell.

30. (Amended) The host cell of claim 25, wherein the host cell lacks a functional protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion, other than Vff2p.

31. (Four Times Amended) A method for increasing cell growth of a yeast host cell, comprising introducing a polynucleotide sequence encoding Vff2p into the cell and culturing the cell, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof.

32. (Thrice Amended) The method for increasing cell growth of a cell according to claim 31, wherein the host cell is cultured under conditions effective to allow expression of the Vff2p.

33. (Four Times Amended) A method for increasing protein secretion from a yeast host cell, comprising introducing a polynucleotide sequence encoding Vff2p into the cell and culturing the cell, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof.

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34. (Twice Amended) The method for increasing protein secretion from a cell according to claim 33, wherein the host cell is cultured under conditions effective to allow expression of the Vff2p.

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36. (Four Times Amended) An isolated vesicular fusion factor 2 protein comprising SEQ ID NO:2 or conservative variations thereof.

37. (Four Times Amended) A method of selecting for a yeast secretory mutant cell containing a polynucleotide sequence encoding a Vff2p operably linked to a promoter, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof, the method comprising growing the yeast secretory mutant cell at a restrictive temperature of about 32-37°C, wherein the restrictive temperature selectively favors mutant cell growth.

38. The method of claim 37, wherein the temperature is at about 37°C.

39. The method of claim 37, wherein the secretory mutant cell is sec17-1, sec18-1, bet1-1, sec22-2, usol-1, pex3-1, sed5-1, cdc48-2, sec7-5, or ypt1-3.28.

40. The method of claim 39, wherein the secretory mutant cell is sec17-1, sec18-1, bet1-1, sec22-2, usol-1, or pex3-1.

41. The method of claim 40, wherein the secretory mutant cell is sec18-1.

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42. (Amended) The method of claim 37, wherein the polynucleotide further comprises a sequence encoding a heterologous target protein operably linked to a second promoter.

46. (Amended) The method of claim 31, 33 or 37, wherein the yeast cell is a *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Pichia pastoris*, *Hansenula polymorpha*, or *Kluyveromyces lactis* cell.

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SEQ
47. An isolated polynucleotide comprising a sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2, wherein the vesicular fusion factor 2 protein increases *Saccharomyces cerevisiae* cell growth or protein expression.

DA
48. An isolated polynucleotide comprising SEQ ID NO:1 encoding a vesicular fusion factor 2 protein that increases *Saccharomyces cerevisiae* cell growth or protein expression.

49. A polynucleotide expression vector comprising a polynucleotide encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2, wherein the vesicular fusion factor 2 protein increases *Saccharomyces cerevisiae* cell growth or protein expression.

50. A recombinant host cell comprising a *Saccharomyces cerevisiae* cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2.

51. A method for increasing cell growth of a *Saccharomyces cerevisiae* host cell, comprising introducing a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2 into the cell and culturing the cell.

52. A method for increasing protein secretion from a yeast host cell, comprising introducing a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2 into the cell and culturing the cell..

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